Modelling alpha-galactosides behavior during cowpea soaking-cooking for nutritional optimization

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Introduction

Cowpea is a legume seed that can be used in over 50 different traditional African dishes in both whole grain and milled forms. Cowpea is rich in proteins (20–25%), starch (45–55%), and micronutrients including vitamins and minerals but also contains some antinutrients such as alpha-galactosides which are responsible for flatulence **(a)**. To be consumed, cowpea often requires soaking and cooking in water **(b)**. During this process, both transport and enzymatic reaction phenomena are occurring that involve alpha-galactosides. An optimized soaking-cooking process could significantly reduce alpha-galactosides content. This target can be achieved through the development of a soaking-cooking simulator using COMSOL Multiphysics® software.

Experimental Set-up

Approximately 30 g of seeds were poured into a beaker filled with 120 mL thermally pre-equilibrated soaking water under agitation. The temperatures and times investigated were 30 °C/0.5–38 h, 60 °C/0.5–14 h and 95 °C/0.25–3 h. For each soaking-cooking experiment (i.e. each couple temperature/time), both seeds and soaking water were collected for the determination of alpha-galactosides content (expressed in g/100g dry basis) by High-performance Anion Exchange Chromatography (HPAE) **(c)**.

Endogenous alpha-galactosidase enzyme was also extracted from crude cowpea flour with 100mM phosphate buffer (pH=6.5), using a cowpea/buffer ratio of 1:4 (w/w) for 0.5 h at 4 °C under gentle stirring. Enzyme activity (expressed in $\mu mol.min^{-1}.mL^{-1}$ of enzyme) was evaluated as the amount of PNP substrate hydrolyzed at 30 °C after 5 min and with or without the standard addition of raffinose, stachyose and verbascose or potential inhibitors such as galactinol and galactose **(d)**.

Both alpha-galactosides kinetics and enzyme activity were thus characterized as a function of soaking-conditions (time and temperature) and a model was adjusted to experimental data.

Model Assumptions

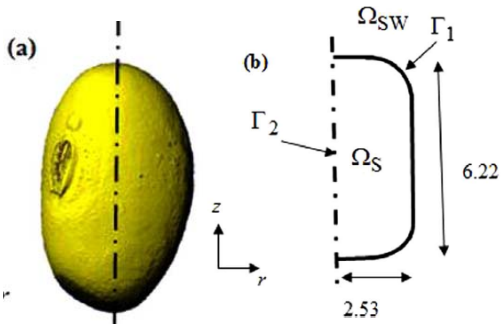

Fig 1: (a) Cowpea seed microtomography. (b) Simplified geometrical representation (Ω_S) (unit: 10^{-3}m)

The cowpea seed is assumed to be a pseudo-ellipsoid (**figure 1**) revolving on a major axis z and having geometrical dimensions that result in a volume equivalent to real seed. During the isothermal soaking-cooking process, the cowpea seed absorbs water, while alpha-galactosides leach out of the seed and/or are degraded or produced in the cowpea seed and in the soaking water. The alpha-galactosides have a sucrose-galactose(n) structure. For verbascose $[Verba]$: n = 3 galactoses $[Gal]$; stachyose $[Stach]$: n = 2 galactoses and raffinose $[Raffi]$: n = 1 galactose. Therefore the degradation of 1 mol of verbascose produces 1 mol of stachyose and the degradation of 1 mol of stachyose produces 1 mol of raffinose.

$$\begin{array}{ccccccc} Verba & \xrightarrow{k_{verba,\Omega}} & Stach & \xrightarrow{k_{stach,\Omega}} & Raffi & \xrightarrow{k_{raffi,\Omega}} & Sucrose \\ \downarrow & & \downarrow & & \downarrow & & \\ Galac & & Galac & & Galac & & \end{array} \quad (1)$$

where $k_{verba,\Omega}$, $k_{stach,\Omega}$ and $k_{raffi,\Omega}$ are respectively the rate constants of verbascose, stachyose and raffinose (s^{-1}) in $\Omega_i = \Omega_S$ or $\Omega_i = \Omega_{SW}$.

The following assumptions (A_i) were formulated for the model:

(A1) A seed is considered to be an homogeneous mixture of insoluble dry matter, water and alpha-galactosides.

(A2) The soaking water is assumed to be perfectly stirred.

(A3) The seed does not swell during soaking-cooking process and the shape of cowpea seed is assumed to be symmetrical along the z axis.

Two domains are considered in the model: a single cowpea seed (Ω_S) and the soaking water medium (Ω_{SW}).

(A4) Except for alpha-galactosides, no dry matter is lost into the soaking water during the process.

(A5) Water as well as the transport of alpha-galactosides are described by Fick's laws of diffusion.

(A6) The degradation of alpha-galactosides follows first-order kinetics in both the seeds and the soaking water (**first modelling approach**).

(A7) The endogenous enzymatic degradation of alpha-galactosides follows Michaelis-Menten law in both the seeds and the soaking water (**second modelling approach**).

Governing Equations

Two modelling approaches were successively developed and integrated into COMSOL Multiphysics version 5.2a, using the coefficient form PDE mode :

- **The first approach** was coupling water transport **(2)** with first-order degradation and diffusion of alpha-galactosides both in seeds **(3)** and soaking water **(4)**:

$$\frac{\partial [W]_{\Omega_S}}{\partial t} - \nabla.\left(D_W \nabla [W]_{\Omega_S}\right) = 0 \qquad \textbf{(2)}$$

Where $[W]_{\Omega_S}$ is the water content in the seed (kg.m^{-3}) and D_W is the apparent water diffusivity (m^2.s^{-1}).

$$\left.\begin{aligned}
&\frac{\partial [Verba]_{\Omega_S}}{\partial t} - \nabla\left(D_{Verba} \nabla [Verba]_{\Omega_S}\right) = -k_{verba,\Omega_S} [Verba]_{\Omega_S} \\
&\frac{\partial [Stach]_{\Omega_S}}{\partial t} - \nabla\left(D_{Stach} \nabla [Stach]_{\Omega_S}\right) = k_{verba,\Omega_S} [Verba]_{\Omega_S} - k_{stach,\Omega_S} [Stach]_{\Omega_S} \\
&\frac{\partial [Raffi]_{\Omega_S}}{\partial t} - \nabla\left(D_{Raffi} \nabla [Raffi]_{\Omega_S}\right) = k_{stach,\Omega_S} [Stach]_{\Omega_S} - k_{raffi,\Omega_S} [Raffi]_{\Omega_S}
\end{aligned}\right\} \qquad \textbf{(3)}$$

$$\left.\begin{aligned}
&V_{\Omega_{SW}} \frac{\partial [Verba]_{\Omega_{SW}}}{\partial t} = J_{Verba} - k_{verba,\Omega_{SW}} [Verba]_{\Omega_{SW}} V_{\Omega_{SW}} \\
&V_{\Omega_{SW}} \frac{\partial [Stach]_{\Omega_{SW}}}{\partial t} = J_{Stach} + k_{verba,\Omega_{SW}} [Verba]_{\Omega_{SW}} V_{\Omega_{SW}} - k_{stach,\Omega_{SW}} [Stach]_{\Omega_{SW}} V_{\Omega_{SW}} \\
&V_{\Omega_{SW}} \frac{\partial [Raffi]_{\Omega_{SW}}}{\partial t} = J_{Raffi} + k_{stach,\Omega_{SW}} [Stach]_{\Omega_{SW}} V_{\Omega_{SW}} - k_{raffi,\Omega_{SW}} [Raffi]_{\Omega_{SW}} V_{\Omega_{SW}}
\end{aligned}\right\} \qquad \textbf{(4)}$$

Where (D_{Verba} , D_{Stach} , D_{Raffi}) are the apparent diffusivities (m^2.s^{-1}) of verbascose, stachyose and raffinose respectively, (k_{verba,Ω_S} , k_{stach,Ω_S} , k_{raffi,Ω_S}) are the rate constants (s^{-1}), J_i are the mass fluxes (kg.s−1) and $V_{\Omega_{SW}}$ (m^3) is the volume and remaining soaking water.

Equations **(2)**, **(3)** and **(4)** have the following initial and boundary conditions (with Verba, Stach and Raffi and boundaries Γ_i shown in figure 1):

$$[W]_{\Omega_S} = [W]^{\infty}_{\Omega_S} \quad \text{on } \Gamma_1 \qquad \textbf{(5)}$$

$$[X]_{\Omega_S} = [X]_{\Omega_{SW}} \quad \text{on } \Gamma_1 \qquad \textbf{(6)}$$

$$\nabla [X]_{\Omega_S} \cdot \vec{n} = 0 \quad \text{on } \Gamma_2 \qquad \textbf{(7)}$$

$$[X]_{\Omega_{SW}} = 0 \qquad \text{in } \Omega_{SW} \text{ for } t = 0 \qquad \textbf{(9)}$$

where $[W]^{\infty}_{\Omega_S}$ is the equilibrium water content at the seed/soaking water interface, and $[X]_{\Omega_i,0}$ are the initial contents of component X in Ω_i (kg.m^{-3}).

The outgoing mass fluxes J_X (kg s^{-1}) of alpha-galactosides crossing the cowpea seed/soaking water interface (Γ_1) can be expressed as:

$$J_X = -AD_X \nabla [X]_{\Omega_S} \qquad \textbf{(10)}$$

where A is the surface area of the seed in contact with the soaking water (m^2).

- **The second approach** was replacing the first-order kinetics by Michaelis-Menten equation:

$$v = \frac{v_{max}[S]}{K_m + [S]} \qquad \textbf{(11)}$$

where v_{max} is the maximum enzymatic activity (µmol.min^{-1}.ml^{-1} of enzyme) and K_m is the Michaelis constant (mM) that can include or not an inhibitory effect.

Numerical solution

The system of four partial differential equations (Eqs (2)−(3)) and of three ordinary differential equations (Eqs (4)) was solved using Comsol Multiphysics™ (version 5.2a, Comsol Inc., Stockholm, Sweden) with the initial conditions given by Eqs. (8) and (9), and boundary conditions given by Eqs. (5)−(7) and Eq. (10) . A 1000-element mesh was created in Comsol. Lagrange polynomials (second order function) were the interpolation functions. The linearized problem was solved by the MUMPS time-dependent solver (Multifrontal Massively Parallel Solver) which implements a parallel distributed LU factorization of large sparse matrixes. The maximum time step was 0.05 s and the Jacobian was updated for each iteration. The typical simulation time was five minutes using a 3.25 Gb free memory (RAM) and 3-GHz Intel core Duo CPU computer (32 bits).

Parameter identification

The three apparent diffusivities (D_i), three rate constants ($k_{i,\Omega}$) and Michaelian parameters (K_m, v_{max}) were simultaneously identified by regression analysis using a Bayesian approach. At 30 °C, both in seeds and soaking water, $k_{raffi,\Omega}$ was set to 2 × $k_{stach,\Omega}$ because of expected higher enzyme affinity for raffinose than for stachyose.

$$[X]_{\Omega_S} = [X]_{\Omega_{S,0}} \quad \text{in } \Omega_S \text{ for } t = 0 \qquad \textbf{(8)}$$

$$C_{ij} = \sum_{u=1}^{n} \left(\frac{\tilde{X}_u^i - \hat{X}_u^i}{\max\{\tilde{X}^i\}} \right) \left(\frac{\tilde{X}_u^j - \hat{X}_u^j}{\max\{\tilde{X}^j\}} \right) \qquad \textbf{(12)}$$

where u is the index of experimental runs ($u = 1, \cdots, 6$) corresponding to the experimental sampling times, $\tilde{X}_u$ are the experimental data points for experimental run u, and $\hat{X}_u$ the values predicted by the model. Here, X are the 3 concentrations in the seed domain (Ω_S): $[Verba]_{\Omega_S}$, $[Stach]_{\Omega_S}$, $[Raffi]_{\Omega_S}$ and 3 concentrations in the soaking water domain (Ω_{SW}): $[Verba]_{\Omega_{sw}}$, $[Stach]_{\Omega_{sw}}$, $[Raffi]_{\Omega_{sw}}$. So, i, j are the indexes of the 6 concentration responses $(i, j = 1, \cdots, 6)$. As shown in Eq. (12), the residuals are evaluated according to the relative difference between the experimental and predicted values. In this approach, not only the sum of squares for each response is taken into account (diagonal elements of matrix C) but also the cross products of the responses (covariance). The model parameters were iteratively adjusted to the goodness-of-merit $\min\{\det(C)\}$ using a minimization procedure of the Nelder-Mead simplex with the "fminsearch" function of Matlab software. The standard deviation of each adjusted parameter was determined via Monte Carlo simulation with 200 draws.

Experimental Results / Simulation Results / Discussion

Figure 2 shows the kinetics of the alpha-galactosides contents in both the seeds and the soaking water at soaking temperatures of 30 °C, 60 °C and 95 °C.

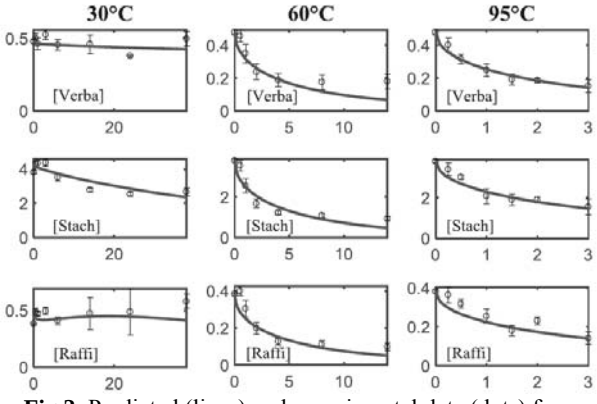

Fig 2: Predicted (lines) and experimental data (dots) for concentrations of raffinose [Raffi], stachyose [Stach], and verbascose [Verba] in cowpea seeds (g/100g on a dry basis) during the soaking-soaking process at 30 °C, 60 °C and 95 °C. Error bars represent standard deviations ($n = 3$).

The initial concentration of verbascose, stachyose and raffinose was 0.48 ± 0.014 g/100g (db), 3.8 ± 0.18 g/100g

In the multi-response approach, the best-fit criterion is the minimization of the determinant of dispersion of matrix C with the elements:

than for verbascose ($0.8 \pm 0.2 \times 10^{-13}$ m^2.s^{-1}). Hence, the adjusted apparent diffusivities were ranked in decreasing order with an increase in the molar mass ($D_{Raffi} > D_{Stach} > D_{Verba}$).

Temperature had a major impact on the diffusion of alpha-galactosides. Shifting from 30 °C to 95 °C resulted in a marked increase in their apparent diffusivities. For instance, verbascose apparent diffusivity increased from $0.8 \pm 0.2 \times 10^{-13}$ m^2.s^{-1} to $6.5 \pm 2.9 \times 10^{-11}$ m^2.s^{-1}. These temperatures are not the usual physiological conditions encountered by seeds and hence result in the degradation of the seed.

At 30 °C, non-null rate constants were found for all the galactosides in the soaking water. The adjusted degradation rate constant was 6.4 fold lower for verbascose (0.5×10^{-4} s^{-1}) than for raffinose. This is logical given the affinity of endogenous alpha-galactosidase enzyme for these different substrates.

At $T = 60$ °C, the adjusted rate constants in the soaking water and for any alpha-galactoside were lower than at 30 °C. For example, the rate constant of stachyose was $0.12 \times 10^{-4} \pm 0.04$ s^{-1} at 60 °C versus $1.6 \times 10^{-4} \pm 0.4$ s^{-1} at 30 °C. This result is in agreement with the optimal temperature range for endogenous alpha- galactosidase activity in legume seeds.

At 95 °C, the rate constants were found to be null both for seed and soaking water. At this temperature, the total alpha-galactoside mass lost by the seeds was fully quantified in the soaking water (i.e. a pure diffusion process).

Table 1. Michaelian parameters (K_m, v_{max}) estimated together for verbascose, stachyose and raffinose (mean values ± standard deviations).

Substrate	Michaelian parameters		RMSE[§] (mM)
	v_{max}(µmol.min^{-1}.mL^{-1})	K_m(mM)	
Verbascose	0.01 ± 0.01	0.25 ± 0.06 [a]	0.01
	0.21	15.9 ± 0.1[b]	0.01
Stachyose	0.22 ± 0.06	4.1 ± 1.8[a]	0.20
	0.21	3.6 ± 0.6[b]	0.23
Raffinose	0.19 ± 0.01	1.2 ± 0.3[a]	0.51
	0.21	1.7 ± 0.3[b]	0.84

Values with different letters (a–b) are significantly different ($p < .05$) in the two conditions (v_{max}free or fixed).
§ RMSE: Root mean square error between experimental and predicted substrate concentration.

(db) and 0.38 ± 0.032 g/100 g (db), respectively. At 30 °C, the adjusted apparent diffusivity was 41 fold higher for raffinose ($9.0 \pm 4.0 \times 10^{-12}$ $m^2.s^{-1}$) than for stachyose ($2.0 \pm 0.6 \times 10^{-13}$ $m^2.s^{-1}$) and 2.6 fold higher for stachyose K_M constant was 3.4 times lower for raffinose (1.2 ± 0.3 mM) than for stachyose (4.1 ± 1.8 mM) and 4.8 times higher than for verbascose (0.25 ± 0.06 mM) (**Table 1**).

The model fitted the experimental data well for all alpha-galactosides considered with an overall RMSE of 0.01 mM for verbascose, 0.2 mM for stachyose (RMSE=0.16 mM at 1.9 mM of stachyose and RMSE=0.23 mM at 11 mM of stachyose), and 0.5 mM for raffinose (RMSE=0.03 mM at 0.15 mM of raffinose; RMSE=0.6 mM at 5.0 mM of raffinose and RMSE=0.9 at 38.0 mM of raffinose) (**Table 1**).

Conclusions

Our study advanced our understanding of the contrasted behavior of alpha-galactosides in cowpea seeds as a function of the soaking-cooking conditions. A 2D axi-symmetric model was developed, considering a single cowpea seed being soaked in a limited amount of water. This approach simultaneously accounts for the diffusion and reaction of alpha-galactosides as well as for its transport of water. At higher temperatures (60 °C and 95 °C), the diffusion process predominated and no thermal degradation was observed. At a low temperature (30 °C), and assuming first-order reactions or Michaelian behaviour, the model satisfactorily described the production and degradation of alpha-galactosides both in the seed and in the soaking water, probably due to endogenous alpha-galactosidase activity. However, in these conditions, the diffusion of alpha-galactosides from the seed to the soaking water was very slow. This simulator could be used to identify optimal soaking-cooking pathways that could minimize alpha-galactosides content in the seed for human consumption. This work is now in progress.

References

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The adjusted v_{max} for raffinose (0.19 ± 0.01 µmol.min^{-1}.mL^{-1} of enzyme) and for stachyose (0.22 ± 0.06 µmol.min^{-1}.ml^{-1} of enzyme) were quite similar, but about 20 times higher than for verbascose (**Table 1**).

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